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CERTIFICATE OF TRANSLATION

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I, JOHN CHARLES McGILLEY, B.A. M.I.T.I., Technical Translator, of c/o Priory Translations Limited, 11, Magdalen Street, Colchester, Essex, England, hereby state:

THAT I am well acquainted with the French and English languages.

THAT I translated the document identified as the Certificate of the French National Institute of Industrial Property and of the certified true copy of the French Patent Application No. 99 07251 filed at the National Institute of Industrial Property on 9th June 1999, from French into English;

THAT the attached English translation is a true and correct translation of French Patent Application No. 99 07251

to the best of my knowledge and belief; and

THAT all statements made of my own knowledge are true and that all statements made on information and belief are believed to be true and further, that these statements are made with the knowledge that wilful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code

JOHN CHARLES McGILLEY

A

FRENCH REPUBLIC

NATIONAL INSTITUTE OF INDUSTRIAL PROPERTY

PATENT OF INVENTION

UTILITY CERTIFICATE - CERTIFICATE OF ADDITION

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The Director General of the National Institute of Industrial Property certifies that the document annexed hereto is the certified true copy of an application for title of industrial property registered at the Institute.

Drawn up in Paris 16th March 2000

For the Director of the National
Institute of Industrial Property

The Head of the Division

[signed]
Martine PLANCHE

NATIONAL INSTITUTE OF INDUSTRIAL PROPERTY

INVENTION PATENT, UTILITY CERTIFICATE

Intellectual Property Code, Book VI

REQUEST FOR GRANT

Confirmation of filing by fax ☐

Date of delivery of documents 9th June 1999 National Registration number 99 07251- Postal code of place of filing 75 INPI PARIS Date of filing 9th June 1999	**1 NAME AND ADDRESS OF APPLICANT OR REPRESENTATIVE TO WHOM ALL CORRESPONDENCE SHOULD BE ADDRESSED** Hoechst Marion Roussel Madame TONNELLIER Marie-José 102, Route de Noisy 93235 ROMAINVILLE CEDEX No. of permanent Power of Attorney: — Ref of correspondent: ML/2518 — Telephone: 0149915410

2. APPLICATION nature of industrial property right

☒ invention patent ☐ divisional application

☐ utility certificate ☐ conversion of a European Patent Application ⇒ **initial application** ⇓

☐ invention patent ☐ utility certificate No. date

Establishment of search report ☐ deferred ☒ immediate

The Applicant requires payment by instalments of the fees ☐ yes ☒ no

Title of the invention (200 characters maximum)

New derivatives of echinocandine, their preparation process and their use as antifungals.

3. APPLICANT (s) SIREN No. 5 5 2 0 8 1 4 7 3 APE-NAF code Name and forenames (underline patronymic name) or name Hoechst Marion Roussel	Legal form Société Anonyme with Board of Directors and Supervisory Board
Nationality FRENCH Complete Address 1, Terrasse Bellini 92800 PUTEAUX	Country FRANCE

4. INVENTOR(S) The inventors are the applicants ☐ yes ☒ no If no, provide a separate designation

5. REDUCTION IN LEVEL OF FEES

6. DECLARATION OF PRIORITY OR REQUEST FOR BENEFIT FROM THE FILING DATE OF A PREVIOUS APPLICATION

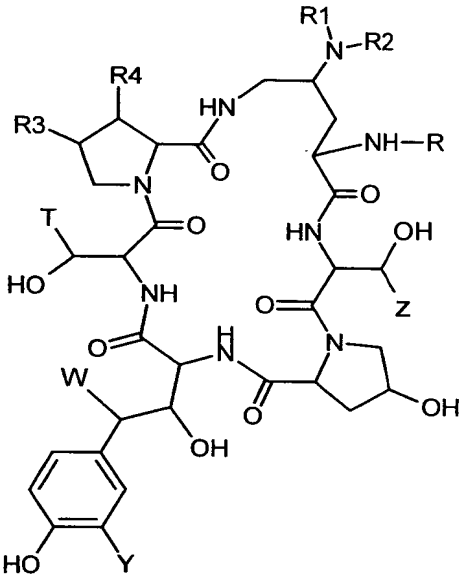
7. DIVISIONS previous to the present application

8. SIGNATURE OF APPLICANT OR REPRESENTATIVE [signed] Marie-José TONNELLIER	SIGNATURE OF RECEPTION OFFICER	SIGNATURE AFTER REG. OF APPLICATION AT N.I.I.P [signed]

New derivatives of echinocandine, their preparation process and their use as antifungals.

The present invention relates to new derivatives of echinocandine, their preparation process and their use as antifungals.

A subject of the invention is, in all possible isomer forms as well as their mixtures, the compounds of formula (I):

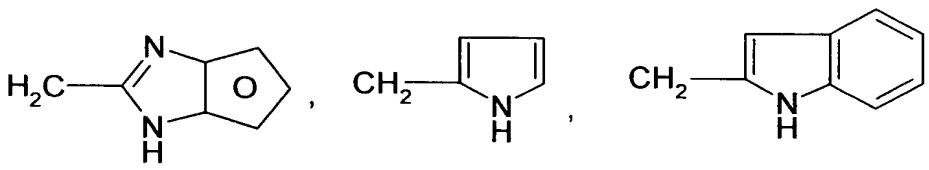


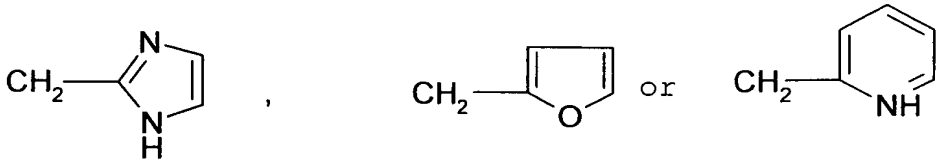
(I)

in which

either R1 represents a hydrogen atom or a methyl radical.

R2 represents a cyclohexyl radical substituted by an amine, a CH2CH2NHCH3 radical, a CH2CHCH3NH2 radical, a





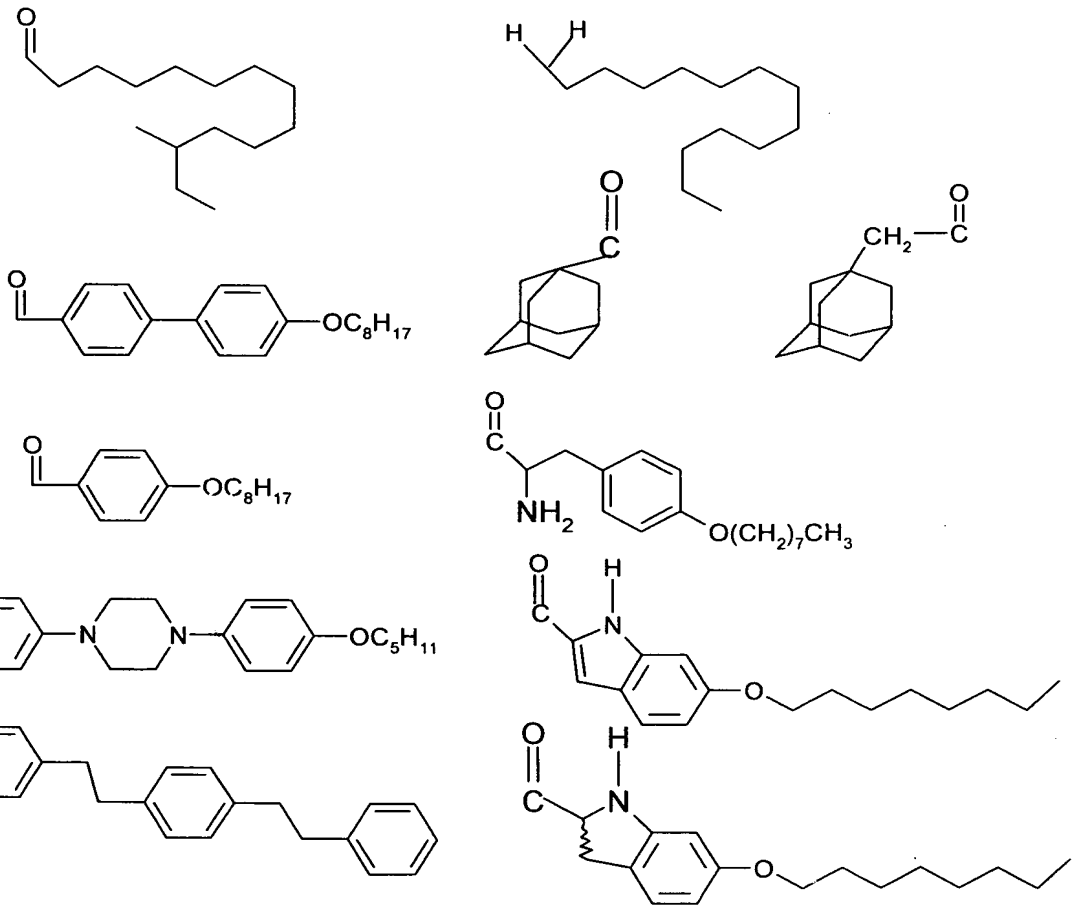
radical, a CHCH3CH2NH2 radical, a -(CH2)aOH radical, a

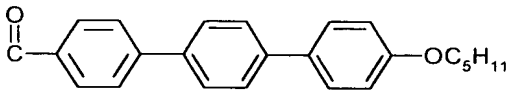
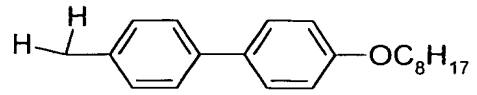
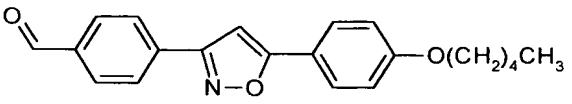
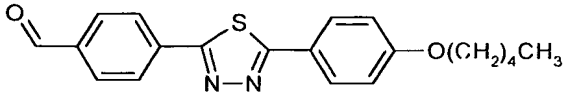
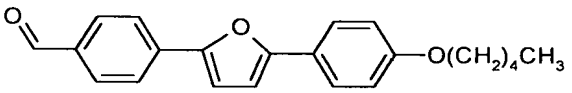
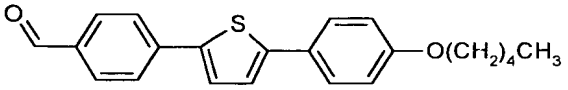
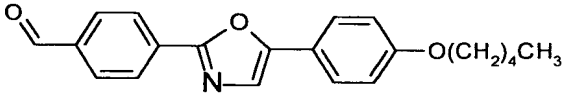
representing an integer comprised between 1 and 8, a (CH2)b-C≡N radical,
b representing an integer comprised between 1 and 8, a CHCH3C6H5 radical, a (CH2)-C(CH3)2NHCOCF3 radical, a CHCH3(CH2)dOH radical, d representing an integer comprised between 1 and 8
or R1 and R2 form together with the nitrogen which carries them a ring with 3, 4 or 5 carbons optionally substituted by an amine
R3 represents a hydrogen atom, a methyl or hydroxyl radical
R4 represents a hydrogen atom or a hydroxyl radical
R represents a linear or branched or cyclic chain containing up to 30 carbon atoms, optionally containing one or more heteroatoms, one or more heterocycles or a linear, branched or cyclic acyl radical containing up to 30 carbon atoms optionally containing one or more heteroatoms and/or one or more heterocycles,
T represents a hydrogen atom, a methyl radical, a CH2CONH2 radical, CH_2C N, a $(CH_2)_2NH_2$ or $(CH_2)_2Nalk^+X^-$ radical, X being a halogen atom and alk an alkyl radical containing up to 8 carbon atoms,
Y represents a hydrogen atom, a hydroxyl radical or a halogen atom or an OSO3H radical or one of the salts of this radical,
W represents a hydrogen atom or an OH radical,
Z represents a hydrogen atom or a methyl radical,
as well as the addition salts with acids of the products of formula (I).

Among the addition salts with acids, there can be mentioned those formed with mineral acids, such as hydrochloric, hydrobromic, sulphuric or phosphoric acid or with organic acids such as formic, acetic, trifluoroacetic, propionic, benzoic, maleic, fumaric, succinic, tartaric, citric, oxalic, glyoxylic and aspartic acids, alkanesulphonic acids, such as methane or ethane sulphonic acid, arylsulphonic acids such as benzene or paratoluene sulphonic acids.

Among the preferred compounds of the invention, there can quite particularly be mentioned the compounds of formula

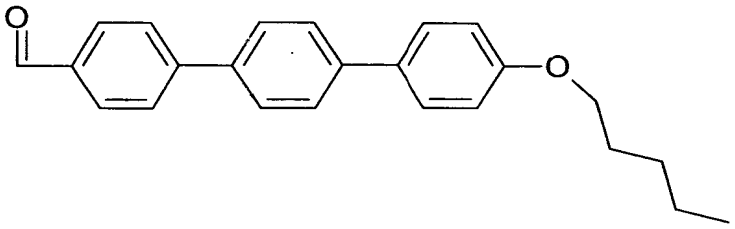
I in which T represents a hydrogen atom, those in which W represents a hydrogen atom, those in which Z represents a methyl radical, those in which Y represents a hydrogen atom, those in which R3 represents a methyl radical, those in which R4 represents a hydroxyl radical, and those in which R represents a



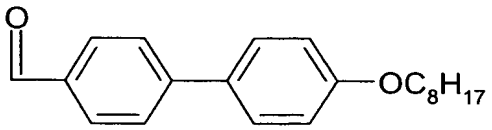








radical.

A most particular subject of the invention is the compounds of formula I in which R represents a

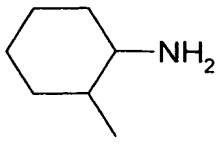


chain or a



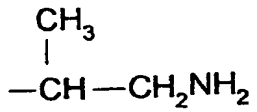
chain.

Among the preferred compounds of the invention, there can be quite particularly mentioned the compounds of formula I in which R1 is a hydrogen atom, those in which R2 is a



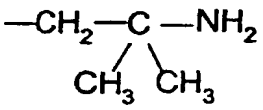
radical,

those in which R2 is a CH2CH(CH3)NH2 radical, a



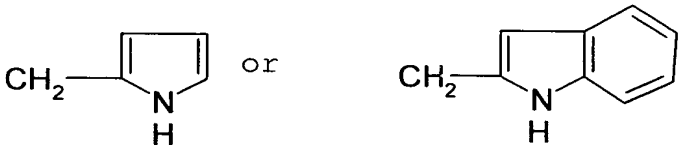
radical

or a



radical

or also those in which R2 is a



radical

A most particular subject of the invention is the compounds of formula (I), the preparation of which is given hereafter in the experimental part and in particular the products of Examples 2 and 3.

The compounds of formula (I) have useful antifungal properties; they are in particular active on Candida albicans and other Candida such as Candida glabrata, krusei, tropicalis, pseudotropicalis, parapsilosis and Aspergillus fumigatus, Aspergillus flavus, Cryptococcus neoformans.

The compounds of formula (I) can be used as medicaments in man or animals, in particular to combat digestive, urinary, vaginal or cutaneous candidosis, cryptococcosis, for example neuromeningeal, pulmonary or cutaneous cryptococcosis, bronchopulmonary and pulmonary aspergillosis and invasive aspergillosis in the immunosuppressed.

The compounds of the invention can also be used in the prevention of mycotic illnesses in the congenital or acquired immunosuppressed.

The compounds of the invention are not limited to a pharmaceutical use, they can also be used as fungicides in

fields other than the pharmaceutical field.

Therefore a subject of the invention is, as antifungal compounds, the compounds of formula (I) as well as their addition salts with acids.

A subject of the invention is also the compounds of formula (I), as medicaments.

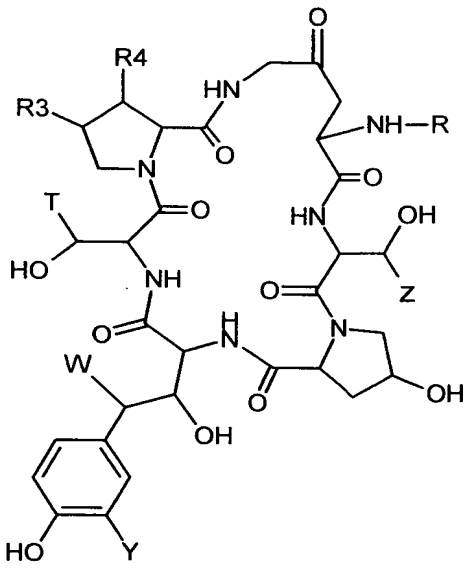
A most particular subject of the invention is the pharmaceutical compositions containing as active ingredient at least one compound of formula (I) or one of its addition salts with pharmaceutically acceptable acids.
These compositions can be administrered by oral, rectal, parenteral route or by local route as a topical application on the skin and mucous membranes, but the preferred route is the buccal route.

They can be solid or liquid and can be presented in the pharmaceutical forms commonly used in human medicine, such as for example, plain or sugar-coated tablets, gelatin capsules, granules, suppositories, injectable preparations, ointments, creams, gels; they are prepared according to the usual methods. The active ingredient or ingredients can be incorporated in the excipients usually used in these pharmaceutical compositions, such as talc, gum arabic, lactose, starch, magnesium stearate, cocoa butter, aqueous or non-aqueous vehicles, fatty matter of animal or vegetable origin, paraffin derivatives, glycols, various wetting, dispersing or emulsifying agents, preservatives.

These compositions can also be presented in the form of a powder intended to be dissolved extemporaneously in an appropriate vehicle, for example apyrogenic sterile water.

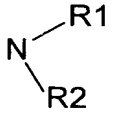
The dose administered is variable according to the illness treated, the patient in question, the administration route and the product considered. It can be, for example, comprised between 50 mg and 300 mg per day by oral route, in adults for the products of Examples 2 and 3.

A subject of the invention is also a preparation process characterized in that a compound of formula (II)



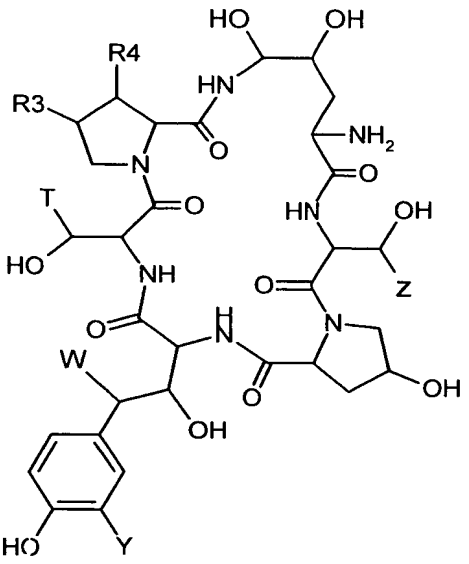
(II)

in which R, R3, R4, T, Y, W and Z retain their previous meaning, is subjected to the action of an amine or an amine derivative capable of introducing

the  radical in which R1 and R2

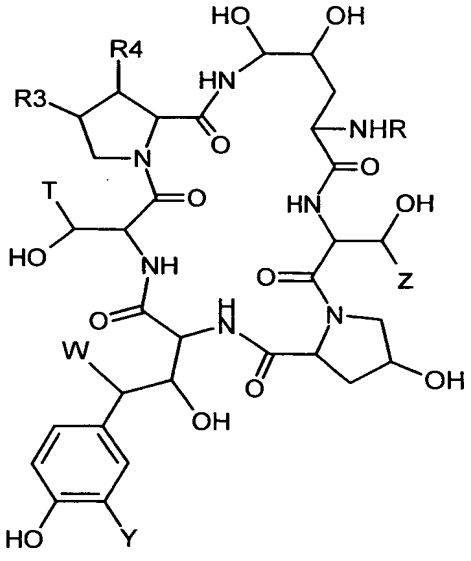
retain their previous meaning and if desired is subjected to the action of a reducing agent
and/or of an amine functionalization agent,
and/or an acid in order to form the salt of the product obtained,
and/or a separation agent of the different isomers obtained,
and the sought compound of formula (I) is thus obtained.

The compounds of formula (II) can be prepared according to a process characterized in that a compound of formula (III)



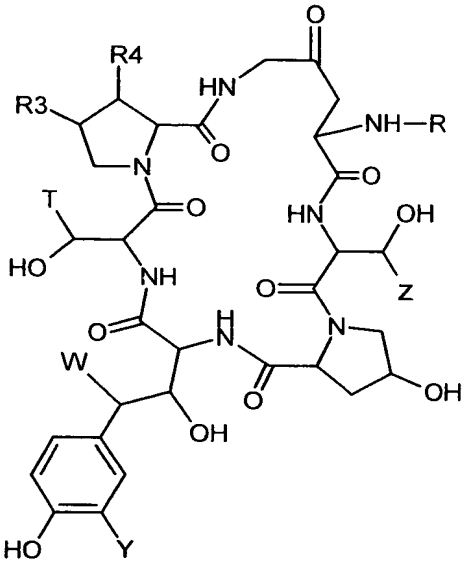
(III)

in which the different substituents retain their previous meaning is subjected to the action of an agent capable of replacing NH2 with NHR, R retaining its previous meaning in order to obtain the compound of formula (IV)



(IV)

which is subjected to the action of trimethylsilyl iodide in order to obtain the corresponding compound of formula (II)



(II)

The following examples illustrate the invention without however limiting it.

Preparation 1: "nucleus" of deoxymulundocandine

2 g of deoxymulundocandine is dissolved in 20 ml of DMSO. This solution is poured into a suspension containing 120 g of Actinoplanes utahensis FH2264 in 870 ml of a KH2PO4, K2HPO4 buffer (pH: 6.8). The reaction mixture is maintained under agitation for 70 hours at 30°C. Filtration is carried out. The mycelium is washed with the phosphate buffer (pH: 6.8). The washing liquids and the filtrate are combined. The product obtained is chromatographed on a DIAION HP 20 resin and a product is obtained which is used as it is hereafter.

EXAMPLE 1:

1-[4-[((2S)-2-amino-2-methylethyl)-amino]-N2-[[4'-(octyloxy)[1,1'-biphenyl]-4-yl]-carbonyl]-L-ornithine]-4-[4-(4-hydroxyphenyl)-L-threonine]5-L-serine-echinocandine B trifluoroacetate (isomer A and isomer B).

Stage A: 1-[(4R,5R)-4,5-dihydroxy-N2-[[4'-(octyloxy)[1,1'-biphenyl]-4-yl]-carbonyl]-L-ornithine]-4-[4-(4-hydroxyphenyl)-L-threonine]-5-L-serine echinocandine B

1- Preparation of the ester

632 mg of 2,3,4,5,6 pentafluorophenol and 695 mg of N,N'-dicyclohexylcarbodiimide are added to 1 g of 4'-octyloxy-

[1,1'-biphenyl]4-carboxylic acid in 22 ml of tetrahydrofuran, followed by agitation for 22 hours at ambient temperature and filtration. The solvents are eliminated under reduced pressure, the residue is taken up in ether, agitated at approximately 35°C, followed by filtration, the solvent is evaporated followed by drying and 1.46 g of expected product is recovered, which is used as it is.

2- Coupling

677 mg of the deoxymulundocandine "nucleus" obtained in Preparation 1 is introduced into 16 ml of DMF. The solution obtained is agitated for 5 minutes and 793 mg of pentafluorophenyl 4'-(octyloxy)-[1,1'-biphenyl]-4-carboxylate obtained above is added. The reaction mixture is maintained under agitation and a nitrogen atmosphere for 24 hours. The reaction mixture is filtered and concentrated. The residue is taken up in ether, triturated, maintained under agitation for 25 minutes, separated, washed with ethyl ether, chromatographed on silica while eluting with a mixture of methylene chloride, methanol, water (86/13/1) then (80/20/1). The sought product is thus obtained. Yield 73%.

Stage B: 1-[N2-[[4'-(octyloxy)-[1,1'-biphenyl]-4-yl] carbonyl]-4-oxo-L-ornithine]-4-[4-(4-hydroxyphenyl)-L-threonine]-5-L-serine-echinocandine B.

311 µl of trimethylsilyl iodide is added to a suspension containing 809 mg of the product of Stage A and 19 ml of acetonitrile. The reaction mixture is maintained under agitation for 15 minutes at 60°C and under a nitrogen atmosphere. The mixture is poured into a saturated solution of sodium thiosulphate followed by evaporation. The residue obtained is chromatographed on silica, eluting with a methylene chloride/methanol/water mixture 86/13/1. The sought product is obtained. Yield 55%.

Stade C: 1-[4-[((2S)-2-amino-2-methylethyl)amino]-N2-[[4'-(octyloxy)[1,1'-biphenyl]-4-yl]-carbonyl]-L-ornithine]-4-[4-(4-hydroxyphenyl)-L-threonine]5-L-serine-echinocandine B trifluoroacetate (isomer A and isomer B).

A solution containing 62.5 mg of (S)-(-)diaminopropane dihydrochloride, 2.25 ml of methanol, triethylamine in order

to obtain a pH of 6, a few grains of activated siliporite and 150 mg of the product of the previous stage is agitated for a few minutes at 20°C. 6 mg of NaBH3CN is introduced. Agitation is carried out for 15 hours at 20°C and after semi-preparative HPLC purification (eluent: CH3CN,H20TFA(50-50-0.02), 11.5 mg of isomer A, 13 mg of isomer B are obtained.

EXAMPLE 2:

1-[4-[[(1H-benzimidazol-2-yl)-methyl)-amino]-N2-[[4"-(pentyloxy)[1,1':4',1"-terphenyl]-4-yl]-carbonyl]-L-ornithine]-4-[4-(4-hydroxyphenyl)-L-threonine]5-L-serine-echinocandine B trifluoroacetate (isomer B).

By operating as previously starting from the nucleus of deoxymulundocandine prepared in Preparation 1 and obtaining 1-[(4R,5R)-4,5-dihydroxy-N2-[[4''-(pentyloxy)[1,1': 4',1''-terphenyl]-4-yl]carbonyl]-L-ornithine]-4-[4-(4-hydroxyphenyl)-L-threonine]-5-L-serine-echinocandine B as intermediate product and the corresponding 4-oxo derivative, the sought product was obtained. Isomer A = 7.4 mg, isomer B = 1.2 mg.

EXAMPLE 3:

Trans 1-[4-[(2-aminocyclo-hexyl)-amino]-N2-[[4"-(pentyloxy)[1,1':4',1"-terphenyl]-4-yl]-carbonyl]-L-ornithine]-4-[4-(4-hydroxyphenyl)-L-threonine]-5-L-serine-echinocandine B trifluoroacetate (isomer A).

By operating as previously, starting from 166 mg of the 4-oxo derivative prepared above and 78 mg of (1R, 2R)1-2-diaminocyclohexane, 462 mg of crude product is obtained which is chromatographed on silica eluting with a methylene chloride, methanol, H2O, acetic acid mixture 86/13/2/1. 100 mg of product is obtained which is purified by semi-preparative HPLC again with a CH3CN/H20/TFA mixture = 50/50/0.1. 55 mg of isomer A, 5.2 mg of isomer B are obtained.

EXAMPLE 4:

1-[4-[(2(S)-aminopropyl)-amino]-N2-[[4"-(pentyloxy)[1,1':4',1"-terphenyl]-4-yl]-carbonyl]-L-ornithine]-4-[4-(4-hydroxyphenyl)-L-threonine]-5-L-serine-echinocandine B trifluoroacetate (isomer A).

By operating as previously, the sought product was obtained.

EXAMPLE: Pharmaceutical composition:

Tablets were prepared containing:

- Product of Example 3 isomer A..... 150 mg
- Excipient s.q.f. ..... 1 g

(Detail of excipient: starch, talc, magnesium stearate).

PHARMACOLOGICAL STUDY

A - Inhibition of the glucan synthase of Candida albicans.

Candida albicans membranes were purified according to the process described by Tang et al Antimicrob. Agents Chemother 35, 99-103, 1991. 22.5 µg of membrane proteins are incubated in a mixture of 2Mm of 14C-UDP glucose (specific activity = 0.34 mCi./mmol, 50 µg of α-amylase, 1Mm of dithiotreitol (DTT), 1Mm EDTA, 100Mm NaF, 7µM of GTP-γ-S, 1M of sucrose and 50Mm of Tris-HCL (pH 7.8) in a volume of 100µl. The medium is incubated at 25°C for 1 hour and the reaction is terminated by adding TCA at a final concentration of 5%. The reaction mixture is transferred onto a pre-humidified glass fibre filter. The filter is washed, dried and its radioactivity is counted.

Mulundocandine is used as a positive control.

Control of the vehicle is carried out with the same quantity of 1% DMSO. The results obtained show that in this test the products of the invention show a good activity in particular the products of Example 3 isomer A.

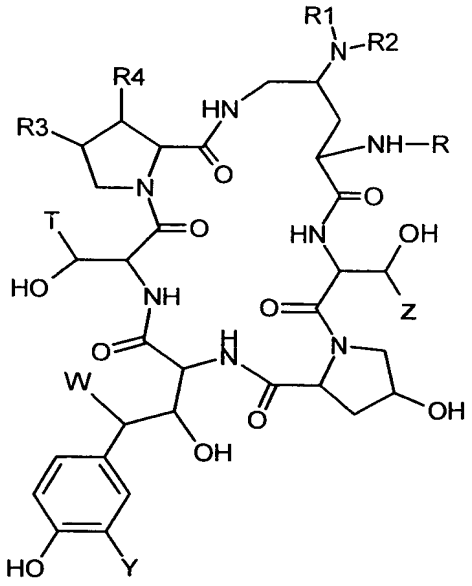
B - activity on the Aspergillus fumigatus enzyme.

The enzyme is prepared according to the process of Beaulieu et al.(Antimicrob. Agents Chenother 38, 937-944, 1994. The protocol used is identical to the protocol described above for the enzyme of Candida albicans except that dithiotreitol is not used in the reaction mixture.

In this test the products show a good activity.

CLAIMS

1) In all possible isomer forms as well as their mixtures, the compounds of formula (I):

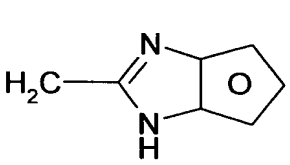
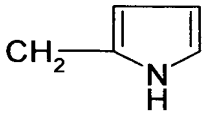


(I)

in which

either R1 represents a hydrogen atom or a methyl radical.

R2 represents a cyclohexyl radical substituted by an amine, a CH2CH2NHCH3 radical, a CH2CHCH3NH2 radical, a


,

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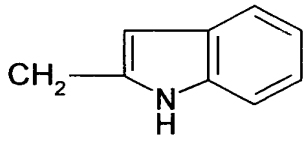
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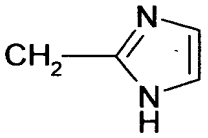
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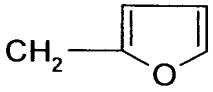
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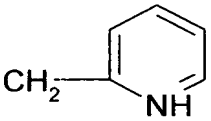


radical, a CHCH3CH2NH2 radical, a -(CH2)aOH radical, a representing an integer comprised between 1 and 8, a (CH_2)b-C≡N radical

b representing an integer comprised between 1 and 8, a CHCH3C6H5 radical, a (CH_2)-$C(CH_3)_2NHCOCF_3$ radical, a

CHCH3(CH2)dOH radical, d representing an integer comprised between 1 and 8

or R1 and R2 together with the nitrogen which carries them form a ring with 3, 4 or 5 carbons optionally substituted by an amine

R3 represents a hydrogen atom, a methyl or hydroxyl radical

R4 represents a hydrogen atom or a hydroxyl radical

R represents a linear or branched or cyclic chain containing up to 30 carbon atoms, optionally containing one or more heteroatoms, one or more heterocycles or a linear, branched or cyclic acyl radical containing up to 30 carbon atoms optionally containing one or more heteroatoms and/or one or more heterocycles,

T represents a hydrogen atom, a methyl radical, a CH_2CONH_2, $CH_2C\equiv N$ radical, a $(CH_2)_2NH_2$ or $(CH_2)_2Nalk^+X^-$ radical, X being a halogen atom and alk an alkyl radical containing up to 8 carbon atoms,

Y represents a hydrogen atom, a hydroxyl radical or a halogen atom or an OSO3H radical or one of the salts of this radical,

W represents a hydrogen atom or an OH radical,

Z represents a hydrogen atom or a methyl radical,

as well as the addition salts with acids of the products of formula (I).

2) The compounds of formula (I) defined in claim 1 in which T represents a hydrogen atom.

3) The compounds of formula (I) defined in claim 1 or 2 in which W represents a hydrogen atom.

4) The compounds of formula (I) defined in any one of claims 1 to 3, in which Z represents a methyl radical.

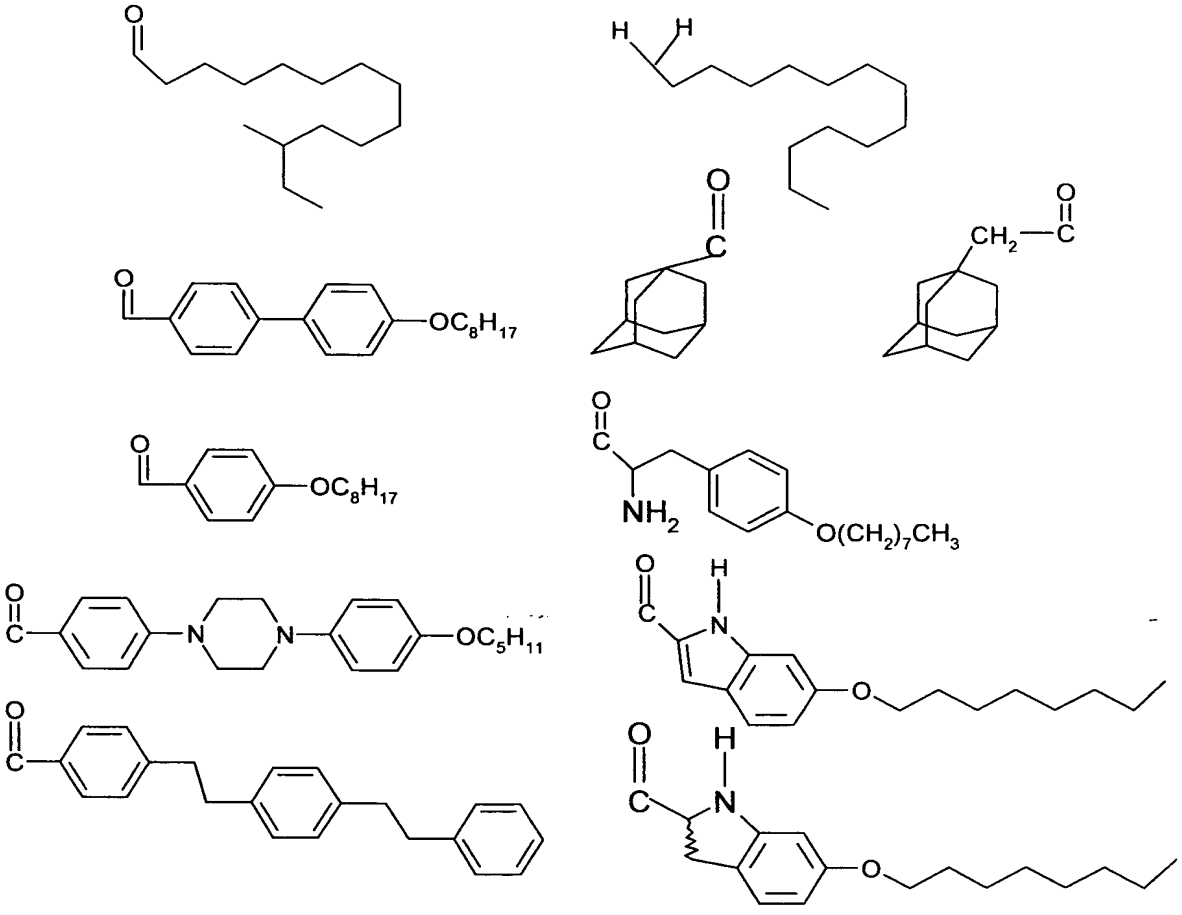
5) The compounds of formula (I) defined in any one of claims 1 to 4 in which Y represents a hydrogen atom.

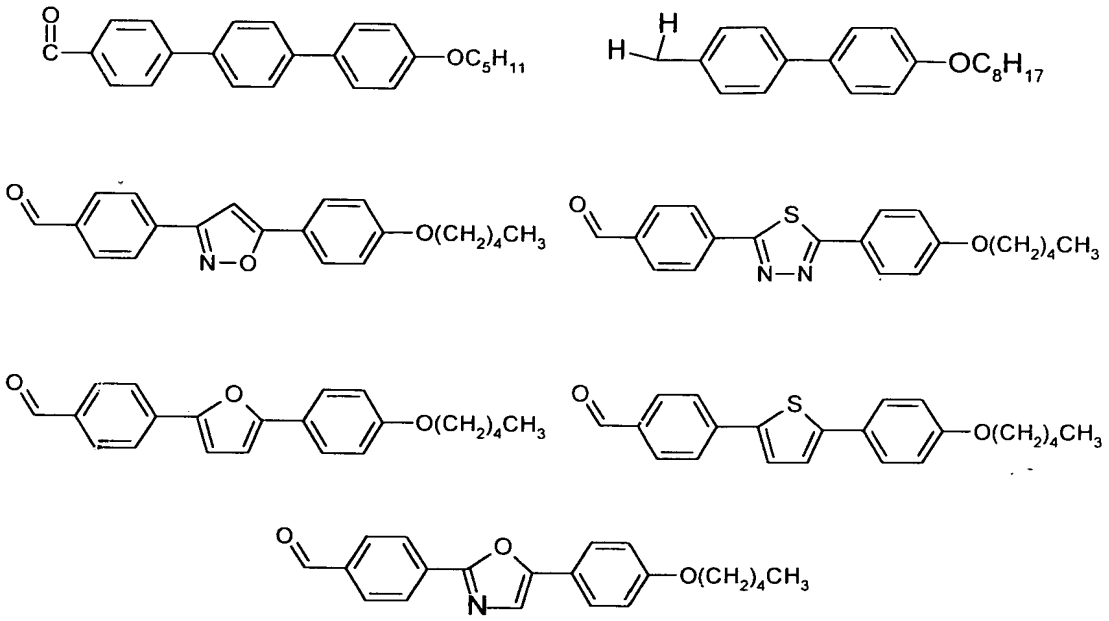
6) The compounds of formula (I) defined in any one of claims 1 to 5 in which R3 represents a methyl radical.

7) The compounds of formula defined in any one of claims 1 to 6 in which R4 represents a hydroxyl radical.

8) The compounds of formula (I) defined in any one of claims 1 to 7 in which R represents a

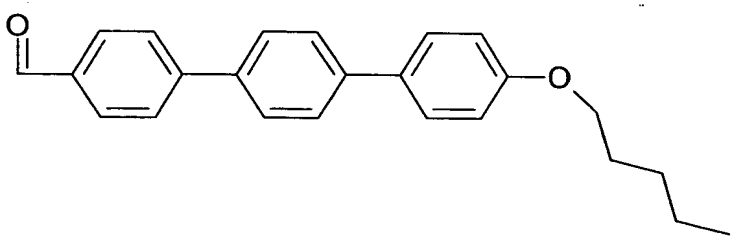
5





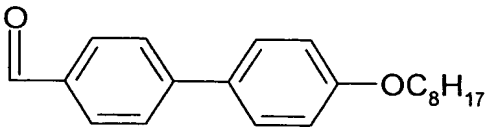
radical.

9) The compounds of formula (I) defined in claim 8, in which R represents a



chain.

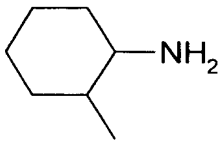
10) The compounds of formula (I) defined in claim 8, in which R represents a



chain.

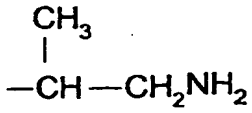
11) The compounds of formula (I) defined in any one of claims 1 to 10 in which R1 is a hydrogen atom.

12) The compounds of formula (I) defined in any one of claims 1 to 11 in which R2 is a

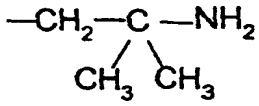


radical.

13) The compounds of formula (I) defined in any one of claims 1 to 11 in which R2 is a CH2CH(CH3)NH2 radical, a

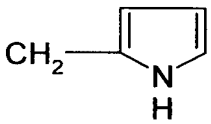


radical or a

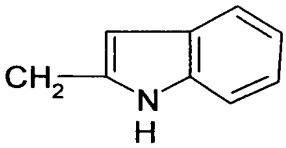


radical.

14) The compounds of formula (I) defined in any one of claims 1 to 11 in which R2 is a

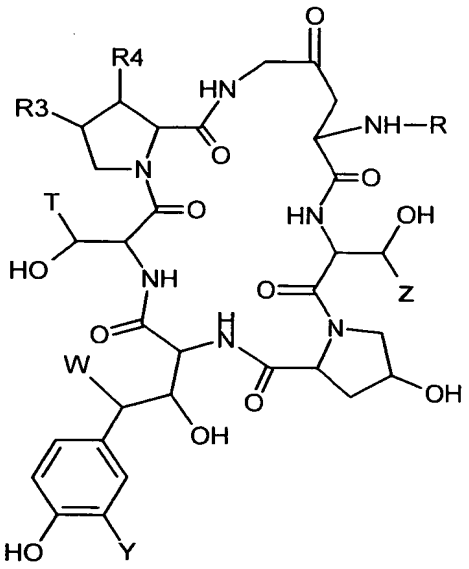


or



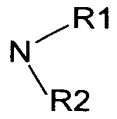
radical.

15 Process for the preparation of compounds of formula (I) defined in any one of claims 1 to 14 characterized in that a compound of formula (II)



(II)

in which R, R3, R4, T, Y, W and Z retain their previous meaning, is subjected to the action of an amine or amine derivative capable of introducing

the  radical in which R1 and R2

retain their previous meaning and if desired to the action of a reducing agent
and/or an amine functionalization agent,
and/or an acid in order to form the salt of the product obtained,
and/or a separation agent of the different isomers obtained,
and the sought compound of formula (I) is thus obtained.

16) As antifungal compounds, the compounds of formula (I) defined in any one of claims 1 to 14, as well as their addition salts with acids.

17) The pharmaceutical compositions containing at least one compound of formula (I) defined in any one of claims 1 to 14 as a medicament, as well as their addition salts with pharmaceutically acceptable acids.